

or ovariectomy (OVX), and administered either vehicle or 30 µg/kg hPTH (1–34aa) by s.c. injection for 5 weeks, n = 10. Serum levels of osteocalcin and CTX-II were measured by specific ELISAs.

**Results:** When stimulated with PTH, the cultured chondrocytes accumulated intracellular cAMP levels significantly ( $P < 0.003$ ) in a dose-dependent manner. The maximum concentration of PTH (100 nM) resulted in a 23-fold increase compared with vehicle control. In the explants cultures of OA articular cartilage, a two-fold increase of PIINP was observed in the supernatant after PTH stimulation when compared to non-stimulated cartilage samples. Furthermore, 10 nM PTH increased incorporation of <sup>35</sup>Sulphate by 40% ( $p = 0.002$ ). The serum level of the bone turnover marker osteocalcin was significantly ( $p < 0.001$ ) elevated in OVX animals that were PTH treated compared to sham and vehicle treated, while the level of serum cartilage degradation marker CTX-II decreased by 30% ( $p < 0.01$ ).

**Conclusions:** The current data strongly suggest that PTH, in addition to osteoblasts and bone turnover, also has direct anabolic effects on chondrocytes and cartilage. We have shown that PTH can not only avert but also facilitate cartilage generation in both *in vitro* and *in vivo* situations. Presented data indicate the potency of PTH and intrigues further investigation of PTH as a potential DMOAD.

#### 161 NMDA RECEPTOR FUNCTION IN OSTEOARTHRITIC CHONDROCYTES IS DEPENDENT ON $\beta 1$ INTEGRINS

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**Purpose:** Osteoarthritis (OA) is associated with abnormal loading of articular joints. In normal joints physiological loading maintains structure and function of cartilage and chondrocytes within. This is mediated by a process known as mechanotransduction whereby the mechanical forces are recognized by the resident chondrocytes and transduced into biochemical and molecular responses. In chondrocytes  $\alpha 5 \beta 1$  integrin is a major mechanoreceptor and activation triggers signaling events and autocrine/paracrine signaling via IL4 which lead to up-regulation of anabolic genes and down-regulation of catabolic activity. In OA however this mechanism is disrupted and mechanically stimulate chondrocytes express pro-inflammatory and catabolic IL1 $\beta$ . The reasons for this are unclear but recent research from our group has raised the possibility that differential involvement of ionotropic glutamate receptors, NMDARs in the mechanotransduction pathway may be important. NMDARs are associated with neurons, although non-neuronal tissues have been shown to express these receptors. In neurons, NMDAR interact with intracellular signaling proteins through the scaffold protein PSD-95. As potential roles for integrins in modifying NMDAR activity have also been shown we have now investigated the relationship between NMDAR signaling in chondrocytes and molecules that are involved in chondrocyte mechanotransduction.

**Methods:** Articular cartilage was removed from OA and chondrocytes released using sequential enzymatic digestion. Chondrocytes were maintained as non-confluent primary monolayer cultures at a concentration of  $5 \times 10^5$  cells/ml. To measure the function of NMDAR cell membrane potentials of OA cells were measured by electrophysiology in resting cells, after treatment (integrin modifying or CD47 antibody (1 µg/ml)) and following stimulation with NMDA (50 µM). Chondrocytes seeded onto cell culture dishes coated with substrates (poly-L-lysine or fibronectin; 10 µg/ml) were used to assess cell membrane potential before and after NMDA treatment. Co-immunoprecipitation of PSD-95 with  $\beta 1$  integrin and CD47 using specific antibodies was analysed by Western blot.

**Results:** Incubation of OA chondrocytes with anti- $\beta 1$  integrin and anti-CD47 (integrin associated protein) function blocking antibodies inhibited the electrophysiological response to NMDA; antibodies to  $\beta 3$ ,  $\beta 5$  and anti- $\alpha$  integrin antibodies had no effect. Integrin dependency of NMDAR signaling with NMDA treatment was analysed; Chondrocytes adherent to poly-L-lysine (integrin independent attachment) showed no electrophysiological response to NMDA while cells adherent to fibronectin and type II collagen ( $\beta 1$  integrin dependent attachment) showed a membrane depolarization. Co-immunoprecipitation experiments of PSD-95 with  $\beta 1$  integrin and CD47 identified that these molecules are physically linked and therefore may be involved in regulating NMDAR function.

**Conclusions:** We identify that NMDAR function in chondrocytes is linked to  $\beta 1$  integrin and CD47, and that the electrophysiological response to NMDA requires integrin dependent cell-matrix interactions. Linkage of  $\beta 1$  integrins and CD47 to PSD-95, possibly as part of a complex that may contain NMDAR, appears to be important in NMDAR activity

in chondrocytes. In neurons studies have shown integrins can be involved in regulating the expression and function of NMDAR, although the mechanisms by which this occurs have yet to be defined. The role of NMDAR in OA chondrocyte/cartilage function is yet to be fully understood, but parallel studies indicate that these receptors may participate in the signal cascade that results in anabolic/catabolic responses to applied mechanical forces.

#### 162 CHONDROCYTES FROM OSTEOARTHRITIS PATIENTS REVERT TO THEIR ORIGIN PHENOTYPE ONCE GROWN ONTO A HYALURONAN-BASED SCAFFOLD

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**Purpose:** To evaluate the expression of some specific extracellular matrix molecules in human chondrocytes from healthy and osteoarthritis cartilages freshly isolated and after their growth onto a hyaluronan-based scaffold used in autologous transplantation procedure.

**Methods:** Chondrocytes were isolated from human articular cartilage obtained from the knees of patients with osteoarthritis and from multiorgan donors. First, the cells were expanded in monolayers and then they were seeded on a hyaluronic-acid derivative scaffold. Constructs were analyzed at 0, 3, 7, 14, 21 and 28 days after seeding. Immunohistochemical analysis for collagen type I, II, proteoglycans, Sox-9, MMP-1, MMP-13, TIMP-1, cathepsin-B was carried out on freshly isolated cells, on cells grown in monolayer culture and after they were grown onto the scaffold. A Real-Time RT-PCR analysis was performed on the constructs to evaluate the expression of the specific genes at the different experimental times evaluated.

**Results:** Chondrocytes freshly isolated from control and OA patient cartilages expressed the same extracellular matrix molecules even if at different amount. These differences, which were appreciable both at protein and molecular levels, were not evident once the cells were grown onto Hyaff®-11 scaffold. In this experimental culture condition the cells derived from control and OA patients showed a significative increase of collagen type II, Sox-9 and aggrecan and a decrease of collagen type I compared to chondrocytes grown in monolayer. On the other hand, MMPs were downregulated in both the cell types evaluated by the specific action of TIMP-1 which was highly expressed at molecular and protein levels in the two groups.

**Conclusions:** The growth of chondrocytes onto Hyaff®-11 membrane seems to erase the differences between the cells derived from normal and OA cartilages. The hyaluronan-based scaffold is able to recapitulate some embryonic events which allow to the expression of the extracellular matrix specific genes and to the production of the appropriate proteins. This is of particular relevance hypothesizing the use of tissue engineering therapeutical approach also in osteoarthritis patients.

#### 163 RELATIONSHIP BETWEEN INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR EXPRESSION AND MICROSCOPIC SCORE IN NORMAL EQUINE JOINTS

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**Purpose:** Osteoarthritis (OA) is a chronic degenerative disease of the articular cartilage which appears to reflect a failure in the attempted repair of the cartilage. Comprehensive data regarding the biological nature of both normal and early OA tissue are lacking. Until such data are available, accurate comparison of variables between cartilage from different joints and potential therapeutic strategies may be compromised. Insulin-like growth factor-1 (IGF-1) is a potential therapeutic agent in OA due to its potent anabolic effect in normal cartilage. In an OA joint, chondrocytes are phenotypically disturbed, and demonstrate altered receptor expression. Disruption in levels of IGF-1 and expression of its receptor (IGF-1R) causes enhanced catabolism of proteoglycans. Therefore the aim of the current study was to investigate IGF-1R expression in macroscopically normal cartilage from grossly normal joints and correlate this with microscopic score, in an attempt to further understand the normal condition of cartilage.

**Methods:** Macroscopically normal cartilage from grossly normal joints was sampled from six sites on the mediolateral metacarpal condyle at distances from the transverse ridge (dashed line in figure 1) of

horses slaughtered for human consumption (grade 0,  $n=6$  mean age  $8.8 \pm 4.7$  yrs).

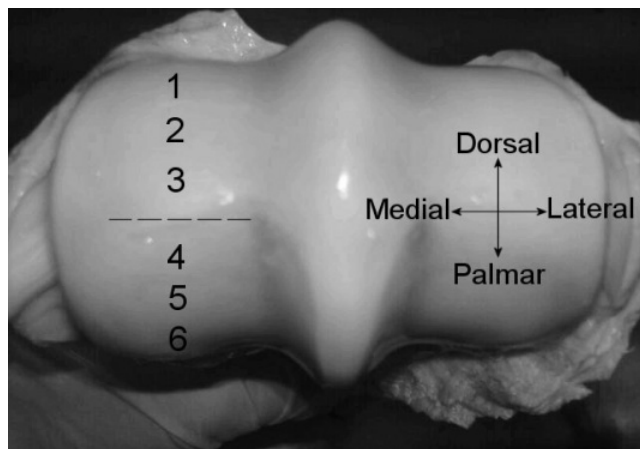


Figure 1: Sampling site of the equine distal metacarpus.

Expression of IGF-1R was determined using immunohistochemistry on  $7\mu\text{m}$  cryostat sections. Mean percentage of cells staining positively for IGF-1R in the superficial zone (%pos.S) and middle+deep zones (%pos.MD), and microscopic (modified Mankin) score were determined for each site. All data were analysed using two-way ANOVA and linear regression.

**Results:** The %pos.S was significantly greater than %pos.MD at four of the six sites investigated ( $P < 0.01$ ), however although there was wide variation there were no significant differences between sites for either variable. The mean (SD) %pos.S and %pos.MD across the joint sites were  $45.03 (\pm 21.71)$  and  $14.87 (\pm 11.28)$ , respectively, while the microscopic score ranged from 1.81–7.38 (mean=4.52). There was a significant positive correlation between both %pos.S and %pos.MD and microscopic score at sites 1 and 2 (Figure 2).

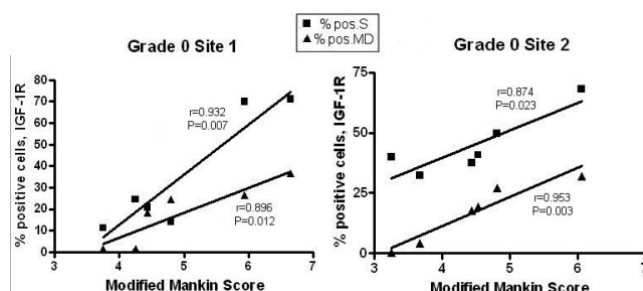


Figure 2: Significant positive correlation between %pos.S, %pos.MD and microscopic score in cartilage from normal joints.

**Conclusions:** The findings of this study indicate that considerable, though statistically insignificant, variations in IGF-1R and microscopic score exist in normal joints. The wide range of microscopic score seen in the normal cartilage used in this study could suggest that some of the scoring criteria of the microscopic scoring system used are transient measures of cartilage condition, or that initial gross grading of cartilage should include additional measures such as Indian Ink stains or the cartilage degeneration index. The unexpected finding of a strong positive correlation between IGF-1R expression and microscopic score in two regions of the joint may indicate that potentially, IGF-1R plays a role in cartilage degradation.

#### 164 A SINGLE BLUNT IMPACT STIMULATED ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASES IN BOVINE CHONDROCYTES

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**Purpose:** Posttraumatic osteoarthritis (PTOA) is one of the most frequent causes of disability after trauma involving weight-bearing joints. Such injuries result in mechanical damage to cartilage surfaces, which appears to induce the progressive degeneration of articular cartilage that

is characteristic of PTOA. The catabolic activity of chondrocytes, the sole cell type in cartilage, is thought to be responsible for this process, but how traumatic injury alters intracellular signaling in chondrocytes resulting in induction of catabolic processes remains unclear. Since two mitogen activated protein (MAP) kinases, JNK and p38, are implicated in many inflammatory or apoptotic pathways, the objective of this study was to determine if these two MAP kinases were activated in chondrocytes by a single blunt impact on cartilage in vitro.

**Methods:** Osteochondral explants were taken from bovine knee joints and impacted with a drop tower (5J) with a 5 mm diameter platen. The cartilage from both the impacted site and the 1.0 mm ring of cartilage around the impact site (annulus) was harvested with biopsy punches and the kinases were extracted in lysis buffer with phosphatase and proteinase inhibitors. The total protein concentration in the lysates was measured by BCA assay. The denatured and reduced lysates were resolved by SDS-PAGE and probed with anti-total and phosphorylated JNK and p38 antibodies.

**Results:** The p38 MAP kinase was activated 20 min after a single blunt impact on cartilage in both impacted site and annulus. The activation diminished at 6 hr but rose up again at 12 hr and 24 hr and more activated p38 was observed in the annulus than in the impacted site (Figure 1). JNK-1 and -2 were activated 20 min after impact (Figure 2). However, the activation of JNKs was not detected in longer term cultures.

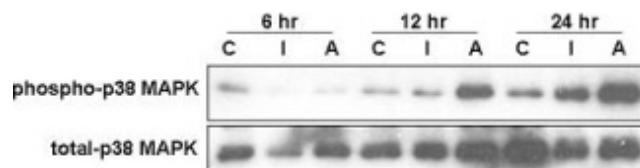


Figure 1: More activated p38 MAPK was detected in the cartilage around the impacted site in a longer term culture. C: non-impacted control; I: impacted; A: annulus of an impacted site.

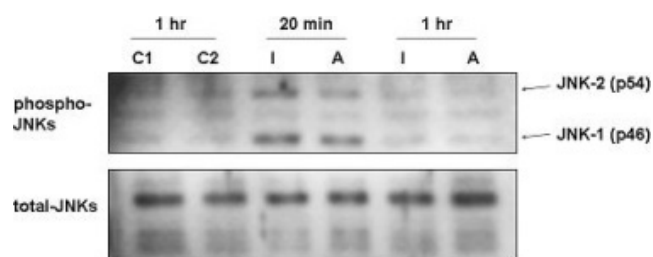


Figure 2: Activation of JNK-1 and JNK-2 20 minutes after a single blunt impact.

**Conclusions:** Our data showed that both p38 and JNKs, the inflammatory MAP kinases, were activated after a single blunt impact to cartilage. This suggests that these two MAP kinases play an important role in posttraumatic chondrolysis. Future work will focus on the application of specific chemical inhibitors of these two MAP kinases, which may be used as interventions to ameliorate posttraumatic osteoarthritis.

#### 165 MELANOCYTE-STIMULATING-HORMONE AFFECTS GENE EXPRESSION IN HUMAN ARTICULAR CHONDROCYTES

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**Purpose:** Melanocortins (MCs) are related peptides and are involved in food intake, energy homeostasis, sexual behaviour, endocrine gland function and inflammatory responses. The natural MCs, adrenocorticotrope hormone (ACTH) and the melanocyte-stimulating hormones ( $\alpha$ -,  $\beta$ -,  $\gamma$ -MSH) derive from proopiomelanocortin (POMC), which is processed by members of the prohormone convertase family. The effects of MCs are transduced by melanocortin receptors (MC-R), a family of five transmembrane G-protein coupled proteins. The MC-system is well described in brain and skin. The additional presence of MC-Rs in rodent and murine osteoblasts and chondrocytes suggests a role for the melanocortin system in cartilage and bone formation.  $\alpha$ -MSH inhibits TNF- $\alpha$  induced MMP-13 expression in human chondrosarcoma cells and, thus may be an inhibitor of MMP-13 mediated collagen degradation. ACTH mediated activation of MC-Rs on knee joint macrophages, reduced IL-1 $\beta$  and IL-6